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THERMAL BEHAVIOR OF SOME N,N-DIMETHYLBIGUANIDE DERIVATIVES DISPLAYING ANTIMICROBIAL ACTIVITY

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N,N-Dimethylbiguanide derivatives (HDMBG)X, where *X*=CH_3COO **(1)**, Cl **(2)** and NO_3 **(3)** respectively, exhibit *in vitro* antimicrobial activity on representative bacterial and fungal strains. The presence of N,N-dimethylbiguanidium ion for all derivatives was evidenced by IR and ^{1}H NMR spectra. Thermal analysis gave information on their decomposition steps and also on the accompanying thermodynamic effects. According to TG and DTG curves processes as melting, oxidative degradation as well as oxidative condensation of –C=N– units occur. The different nature of the anions results different melting points. Paracyanide formation at various condensation degrees was observed.

Keywords: *antimicrobial activity, N,N-dimethylbiguanide, paracyanide, thermal behaviour*

Introduction

N,N-dimethylbiguanide (Metformin) is known as an agent that decreases the glucose level and also acts as analgesic, antimalarial and antimetabolite for organisms that inhibit the metabolism of folic acid [1–3]. As insulin sensitise, Metformin acts predominantly on the liver where it suppresses glucose release. Moreover biguanide derivatives possess groups able to generate hydrogen bonding, which play a very important role in the interactions with biomolecules [4]. Recently it was also showed that some dimethylbiguanide complexes present antimicrobial activity [5, 6] and also exhibit an interesting thermal behaviour [5–8].

In order to modulate the biological activity of N,N-dimethylbiguanide (DMBG) and to correlate this activity both with structure and thermal stability a series of derivatives of type (HDMBG)X (**(1)** *X*: CH_3COO; **(2)** X: Cl and **(3)** *X*: NO_3) have been synthesised and characterised by elemental, IR and ^{1}H NMR spectroscopy.

The in vitro antimicrobial testing was performed against bacterial strains, Gram-positive (*Bacillus subtilis, Listeria monocytogenes*, *Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enteritidis*), as well against fungal strains (*Candida sp.*), using both reference and clinical, multidrug resistant strains.

Thermal behaviour of these derivatives was investigated in order to evidence the decomposition steps and also the corresponding thermodynamic effects. According to TG and DTG curves processes as melting, oxidative degradation and oxidative condensation of –C=N– units occur. Some paracyanide species formed during thermal degradation were isolated and characterised.

Experimental

Synthesis of the compounds

All reagents were of commercial analytical quality and have been used without further purification.

The compound (HDMBG)(CH_3COO) **(1)** was obtained adding a solution of 10 mmoles (HDMBG)Cl in 50 cm^3 water to 100 cm^3 aqueous solution containing 5 mmoles $Hg(CH_3COO)_2$ under continuous stirring. The white sparingly soluble compound ($HgCl_2$) formed immediately was filtered off and the solution was kept at room temperature for several weeks until the white crystals of compound were formed. The crystals were filtered, washed with ethanol and air-dried.

Analysis found: C, 38.11; H, 7.86; N, 37.12%; calculated for $C_6H_{15}N_5O_2$: C, 38.08; H, 7.99; N, 37.01%; IR (KBr pellet), cm^{-1}: $\nu_{as(NH_2)}$, 3313vs; $\nu_{s(NH_2)}$, 3106vs; $\nu_{as(COO)}$, 1672vs; $\nu_{(C=N)}$, 1583vs;

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$\delta_{as(NH_2)}$, 1549vs; $\nu_{s(COO)}$, 1487vs; $\delta_{s(NH_2)}$, 1405m; $\gamma_{(C-N)}$, 1280w; $\rho_{(CH_3)}$, 728w; $\delta_{(COO)}$, 652m; NMR: ^{1}H(d_6-DMSO) δ, 1.93 (s, 3H, CH_3); 3.04 (s, 6H, CH_3); 6.62 (s, 4H, NH_2), 7.39 (s, 2H, NH).

(HDMBG)Cl **(2)**: IR (KBr pellet), cm^{-1}: $\nu_{as(NH_2)}$, 3375vs; $\nu_{s(NH_2)}$, 3297s; $\nu_{(NH)}$, 3175vs; $\nu_{(C=N)}$, 1629vs; $\delta_{as(NH_2)}$, 1578vs; $\delta_{s(NH_2)}$, 1478m; $\nu_{(C-N)}$, 1277w, 1235w; $\rho_{(CH_3)}$, 729w; ^{1}H NMR (d_6-DMSO): δ 2.92 (s, 6H, CH_3), 6.80 (s, 4H, NH_2), 7.22 (s, 2H, NH).

(HDMBG)NO_3 **(3)**: IR (KBr pellet), cm^{-1}: $\nu_{as(NH_2)}$, 3336vs; $\nu_{s(NH_2)}$, 3207vs; $\nu_{(C=N)}$, 1638vs; $\delta_{as(NH_2)}$, 1571vs; $\delta_{s(NH_2)}$, 1494m; $\nu_{3(NO_3)}$, 1313s, 1111w; $\nu_{1(NO_3)}$, 1182w; $\nu_{2(NO_3)}$, 855w; $\nu_{4(NO_3)}$, 725m; ^{1}H NMR (d_6-DMSO): δ 2.93 (s, 6H, CH_3), 6.59 (s, 4H, NH_2), 7.24 (s, 2H, NH).

The syntheses and structural data for (HDMBG)X (**(2)** *X*: Cl and **(3)** *X*: NO_3)) were reported elsewhere [9, 10]. The composition of compounds has been confirmed by chemical analyses.

Methods

Chemical analysis of carbon, nitrogen and hydrogen has been performed using an EA 1110 analyzer.

IR spectra were recorded in KBr pellets with a Bio-Rad FTIR 135 spectrometer in the range 400–4000 cm^{-1}.

^{1}H NMR spectra were recorded on a Bruker Avance DPX250 spectrometer (working frequency 250 MHz) at 25°C. Chemical shifts were measured in parts per million from internal standard tetramethylsilane.

The in vitro biological activity screening tests of the N,N-dimethylbiguanide derivatives were performed using microbial inoculum ($1.5 \cdot 10^8$ UFC/mL) prepared from Gram positive and Gram negative bacteria, reference and environmental strains (*Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enteritidis*), as well from fungal strains (*Candida sp.*). The antibiogram technique, namely the liquid medium dilution method was used for determining minimum inhibitory concentration (M.I.C., μg mL^{-1}). Stock solutions were prepared by dissolving the compounds in DMF. The standard inoculum of microbial strains was inseminated in a discontinuous gradient of concentration, represented by the tested compounds, in tubs containing nutrient broth Mueller-Hinton. This mixture was incubated at 37°C for 24 h.

The heating curves (TG, DTA and DTG) were recorded in a static air atmosphere using a MOM (Hungary) derivatograph, type Paulik–Paulik–Erdey with a sample mass between 32–50 mg over the temperature range of 20–1000°C, at a heating rate of 10°C min^{-1}.

Results and discussion

Physico-chemical characterisation of compounds

In this paper, we report the preparation, physico-chemical and biological characterisation of some N,N-dimethylbiguanidium derivatives of type (HDMBG)X (**(1)** *X*: CH_3COO; **(2)** *X*: Cl and **(3)** *X*: NO_3). The major goal of this paper was to evidence the thermal behaviour of these compounds that also present *in vitro* an antimicrobial activity.

The major IR spectral features of compounds presented at experimental part indicate that in all spectra appear the characteristic bands of biguanidium moiety [11].

In the range characteristic for the NH vibrations, the spectra of N,N-dimethylbiguanide derivatives display two components, which could be associated with the presence of primary amine group. For **(2)** the additional band, at 3297 cm^{-1}, could be assigned to imine group. The bands corresponding to the vibration modes of the NH_2 group are significantly shifted towards lower wavenumbers in the spectra of the compounds **(1)** and **(3)**. This information suggests that the hydrogen bonds with anions are stronger for these derivatives. This is not surprising having in view that oxygen is more electronegative than chlorine. In the range characteristic for the $\nu_{(C=N)}$, $\delta_{as(NH_2)}$, and respectively $\delta_{s(NH_2)}$ vibrations [12] the spectra display three bands.

For **(1)** the acetate involves in hydrogen bonds generates a C_2 symmetry that leads to two strong bands at 1672 ($\nu_{as(COO)}$) and 1487 ($\nu_{s(COO)}$) cm^{-1}. The bands position is similar with that observed for complexes containing acetate as bridge and display the same symmetry [13]. For compound **(3)** the nitrate anion generates new bands, their position and intensity being also similar with those of complexes that contain this ion as bidentate (Fig. 1a) [14].

This arises from the fact that both species contain nitrate ion in C_{2v} symmetry. This behaviour is generated as in the case of compound **(1)** by the hydrogen bonds realised with some amine group of N,N-dimethylbiguanidium cation.

The ^{1}H NMR spectra of all N,N-dimethylbiguanidium derivatives exhibit a pattern correlated with the isolated methyl protons in the region 2.9–3.1 ppm. The ^{1}H NMR spectrum of N,N-dimetylbiguanidium chloride is shown in Fig. 2. Additional resonance arise from the amine and imines protons at δ: 6.59–6.80 and δ: 7.22–7.39 ppm respectively. The spectrum of **(1)** display an additional signal at

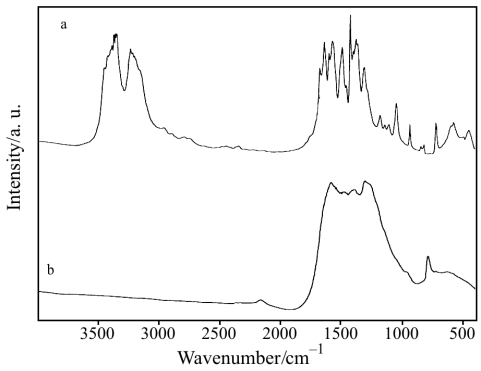

Fig. 1 IR spectra of the a – $(HDMBG)NO_3$ and b – intermediate formed at 320°C

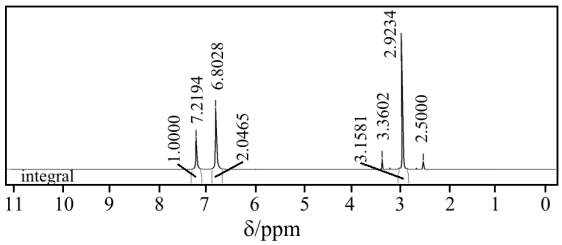

Fig. 2 ^{1}H NMR spectrum of the (HDMBG)Cl

1.93 ppm associated with the acetate presence. Again the downfield shift of resonance assigned to amine group for compounds **(1)** and **(3)** can be correlated with stronger hydrogen bonds.

Biological activity

Antibacterial activity of the N,N-dimethylbiguanide derivatives have been carried out on Gram positive and Gram negative bacteria, reference and clinical strains (*Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enteritidis*), as well on fungal strains (*Candida sp.*) using liquid medium dilution method. The values of minimum inhibitory concentration (M.I.C., μg mL^{-1}) are presented in Table 1.

It was observed that the DMF do not influence the antimicrobial activity of the tested compounds at the working concentrations. Except the hydrochloride derivative, the activity is present at a low level. The M.I.C. values indicate a better activity against the Gram-negative strains, comparatively with Gram-positive bacterial strains, except that on *Bacillus subtilis*. It is to be pointed that compound **(2)** inhibits at a low M.I.C. the *Pseudomonas aeruginosa* growth, that usually is very resistant to antibiotics, including carbapenems, which are β-lactame derivatives.

Thermal behaviour of compounds

The results concerning the thermal decomposition of the compounds evidenced a similar thermal behaviour and the same general aspect of the DTG, DTA and TG curves. Before the thermal decomposition, the compound **(1)** melting at 125°C was observed (Table 2). The melting is immediately followed by the thermal decomposition that starts in the first step with CH_3COOH elimination and partial oxidative degradation of DMBG, which generates an intermediate until 300°C. The nature of this intermediate has been established by chemical analysis and IR spectra where the characteristic acetate bands disappeared. The new bands which appear at 1605 ($\nu_{(C=N)}$), 1331($\nu_{(C=C)}$) and 805 cm^{-1} ($\nu_{(C-C)}$) are characteristic for paracyanide. This step is an overlapping of at least two processes as both DTA and DTG curves indicate. The third step corresponds to paracyanide transformation that involves a depolymerisation together with an oxidative degradation (according to DTA and DTG curves).

These two decomposition steps can be observed also for compound **(2)** (Fig. 3) after the melting at 180°C. Comparing with **(1)** the first step is more complex comprising four processes (two endo and two exo) according to DTA. The paracyanide formed after this step suffers also depolymerisation and oxidative degradation in the last step.

The thermal behaviour of **(3)** is similar with **(1)** as is shown in Fig. 4. After melting at 135°C, the first step that occurs with maximum rate at 230°C comprise two processes as both DTA and DTG curves indicate. Paracyanide species, having a certain polymerisation degree, is formed after this step as indicate the chemical analysis and IR spectrum (Fig. 1b). The characteristic bands of paracyanide are observed at 1603, 1322 and 807 cm^{-1} together with the absence

Table 1 The minimum inhibitory concentration (M.I.C., mg mL^{-1}) of dimethylbiguanide derivatives

Compounds	Microbial strains						
	Bacillus subtilis	*Staphylococcus aureus*	*Listeria monocyt.*	*Escherichia coli*	*Salmonella sp.*	*Pseudomonas aeruginosa*	*Candida albicans*
$(HDMBG)CH_3COO$	>1024	>1024	>1024	>1024	>1024	>1024	>1024
(HDMBG)Cl	128	>1024	>1024	128	128	128	>1024
$(HDMGB)NO_3$	>1024	>1024	>1024	>1024	>1024	>1024	>1024

Table 2 Thermal behaviour of the N,N-dimethylbiguanide derivatives

Compound	Step	Thermal effect	Temp. interval/°C	Δm_{exp}/%	Δm_{calc}/%
(HDMBG)CH_3COO **(1)**	1	endothermic	125 (m. p.)*	0	0
	2	exothermic	190–300	80.32	80.74
	3	exothermic	300–585	19.68	19.26
(HDMBG)Cl **(2)**	1	endothermic	180 (m. p.)*	0	0
	2	exothermic	200–360	77.72	78.01
	3	exothermic	360–690	22.28	21.99
(HDMBG)NO_3 **(3)**	1	endothermic	135 (m. p.)*	0	0
	2	exothermic	180–305	80.92	81.04
	3	exothermic	305–580	19.08	18.95

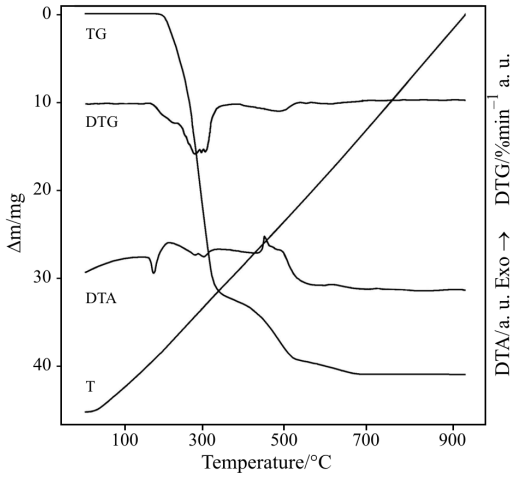

Fig. 3 TG, DTG and DTA curves of (HDMBG)Cl

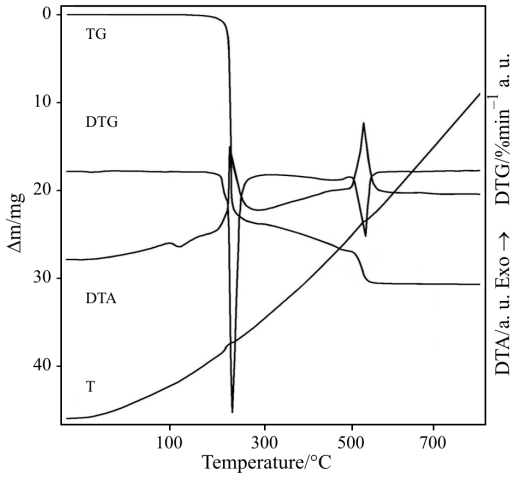

Fig. 4 TG, DTG and DTA curves of (HDMBG)NO_3

of the nitrate bands. The third step corresponds to the transformation of the paracyanide as DTA and DTG indicate. Two processes corresponding to the depolymerisation and oxidative degradation of the paracyanide occur in this last step. The decomposition steps are not well delimited.

It can be observed that the different nature of the anion generates the different melting point. The higher melting point observed for compound **(2)** could be explained through a higher electrostatic interaction between N,N-dimethylbiguanidium cation and anions. Thus chloride anion having the smaller ionic radius generates the strongest attraction with cation. The compounds are very stable and the thermal decomposition begins at about 200°C. The decomposition of the compounds starts at high temperature that is a clue of the stabilisation through hydrogen bonds network.

Conclusions

A series of N,N-dimethylbiguanidium derivatives were characterised in order to obtain new effective antimicrobial agents with a large spectrum of biological activity.

This kind of compounds could be included in a polymeric matrix either by dispersion or by direct polycondensation with suitable organic monomers. Considering that these processes are usually performed at heating, it is important to investigate their thermal behaviour in order to evidence the thermal stability ranges.

The spectroscopic studies revealed the presence of N,N-dimethylbiguanidium ion.

The minimum inhibitory concentration (M.I.C.) indicates that the compound **(2)** exhibit a good antimicrobial activity, which is not influenced by the solvent nature.

The compounds melt before thermal degradation, which occur in two steps and comprises acids elimination, oxidative N,N-dimethylbiguanide degradation as well as paracyanide depolymerisation.

Acknowledgements

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